TOOTH AGENESIS IN THAI POPULATION: PREVALENCE, CHARACTERISTICS AND ITS GENETIC VARIANT IN A THAI FAMILY

Miss Charinya Kanchanasevee

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การศึกษาความชุก และลักษณะเฉพาะของภาวะฟันหายในผู้ป่วยไทย และการเปลี่ยนแปลงทางพันธุกรรมที่เกี่ยวข้องกับภาวะฟันหายในครอบครัวคนไทย

น.ส.ชรินญา กาญจนเสวี

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Thesis Title	TOOTH AGENESIS IN THAI POPULATION: PREVALENCE, CHARACTERISTICS AND ITS GENETIC VARIANT IN A THAI FAMILY
By	Miss Charinya Kanchanasevee
Field of Study	Geriatric Dentistry and Special Patients Care
Thesis Advisor	Associate Professor THANTRIRA PORNTAVEETUS, D.D.S., Ph.D
Thesis Co Advisor	Assistant Professor PISHA PITTAYAPAT, D.D.S., Ph.D

Accepted by the FACULTY OF DENTISTRY, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF DENTISTRY

(Assistant Professor SUCHIT POOLTHONG, D.D.S., Ph.D)

THESIS COMMITTEE

..... Chairman

(Assistant Professor ANJALEE VACHARAKSA, D.D.S., Ph.D)

..... Thesis Advisor

(Associate Professor THANTRIRA PORNTAVEETUS, D.D.S., Ph.D)

..... Thesis Co-Advisor

(Assistant Professor PISHA PITTAYAPAT, D.D.S., Ph.D)

..... External Examiner

(Assistant Professor Paramaporn Chiewpattanakul Kaewmanee, D.D.S., Ph.D)

ชรินญา กาญจนเสวี : การศึกษาความชุก และลักษณะเฉพาะของภาวะฟันหายในผู้ป่วยไทย และการเปลี่ยนแปลงทางพันธุกรรมที่เกี่ยวข้องกับภาวะฟันหายในครอบครัวคนไทย . (TOOTH AGENESIS IN THAI POPULATION: PREVALENCE, CHARACTERISTICS AND ITS GENETIC VARIANT IN A THAI FAMILY) อ.ที่ปรึกษาหลัก : รศ. ทญ.ดร.ทัณฑิริรา พรทวีทัศน์, อ.ที่ปรึกษาร่วม : ผศ. ทญ.ดร.พิสชา พิทยพัฒน์D.D.S., Ph.D

วัตถุประสงค์ เพื่อศึกษาความชุก และลักษณะเฉพาะของภาวะฟันหายในผู้ป่วยไทย และศึกษาเปลี่ยนแปลงทางพันธุกรรมที่เกี่ยวข้องกับภาวะฟันหายในครอบครัวคนไทย

วิธีการ ศึกษาภาพถ่ายรังสีพานอรามิกของกลุ่มผู้ป่วยอายุระหว่าง 15 - 20 ปี ที่ถูกถ่ายในปีพ.ศ. 2560 ณ คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และทำการบันทึกข้อมูลของกลุ่มตัวอย่าง อายุ เพศ และลักษณะฟันของผู้ป่วย ศึกษาความชุกของสภาวะฟันหายแต่กำเนิด โดยใช้การทดสอบทางสถิติเปรียบเทียบความแตกต่างในด้านเพศ ขากรรไกรบน-ล่าง ข้างซ้าย-ขวา และจำนวนฟันที่หาย จากนั้นเก็บตัวอย่างเลือดปริมาณ 3 มิลลิลิตร จากผู้ป่วยที่ให้คำยินยอม เพื่อทำการสกัดดีเอ็นเอและวิเคราะห์การเปลี่ยนแปลงทางพันธุกรรม

ผลการศึกษา ความชุกโดยรวมของผู้ป่วยภาวะฟันหายไม่รวมฟันกรามซี่ที่สามคิดเป็น 9.23% หรือผู้ป่วยจำนวน 101 คน จากจำนวนผู้ป่วยทั้งหมด 1090 คน โดยความชุกภาวะฟันหายในเพศหญิง (9.95%) สูงกว่าเพศชาย (8.23%) เมื่อแบ่งตามประเภทของฟันที่หายไป พบว่า ฟันกรามน้อยล่างซี่ที่สองหายไปมากที่สุด (25.00%; n = 55) รองลงมาคือ ฟันตัดซี่ข้างด้านล่าง (23.28%; n = 51) และฟันตัดซี่ข้างด้าน (15.91%; n = 35) สำหรับซี่ฟันที่หายไปมากที่สุดได้แก่ ฟันตัดซี่ข้างด้านล่างขวา (15.00%; n = 33) รองลงมา ได้แก่ ฟันกรามน้อยล่างขวาซี่ที่สอง (12.70%; n = 28), ฟันกรามน้อยล่างซ้ายซี่ที่สอง (12.27%; n = 27) และ ฟันตัดซี่ข้างด้านบนขวา (8.64%, n = 19) เมื่อแบ่งตามจำนวนฟันที่หายไป พบมีผู้ป่วยฟันหายจำนวนหนึ่งซี่คิดเป็น 43.56% จำนวนสองซี่คิดเป็น 41.58% จำนวนสามซี่คิดเป็น 4.95% และมีฟันหายมากกว่าหกซี่คิดเป็น 5.94% เมื่อแบ่งตามข้างเละขากรรไกรที่ฟันหายพบว่า ฟันหายที่ด้านขวา (54.09%) พบบ่อยกว่าด้านซ้าย (45.91%) และพบในขากรรไกรล่าง (61.82%) มากกว่าขากรรไกรบน (38.18%) สำหรับการศึกษาการเปลี่ยนแลงทางพันธุกรรม พบการกลายพันธุ์ของยีน *WNT10A* มากที่สุด โดยพบในผู้ป่วย 3 รายจากจำนวนผู้ป่วยทั้งหมด 11 รายคิดเป็น 27.27% โดยผู้ป่วยทั้ง 3 ราย พบการหายของซี่ฟันจำนวน 9 ซี่ 8 ซี่ และ 2 ซี่ ตามลำดับ โดยพบการกลายพันธุ์เฮเทอโรไซกัสแบบซ้ำ c.916_918dupAAC (p.Asn306dup) ในยีน *WNT10A* ในผู้ป่วยรายแรก ขณะที่ผู้ป่วยรายที่สองพบการกลายพันธุ์แบบโฮโมไซกัสที่มีการเปลี่ยนเบส c.511C> T (p.Arg171Cys) ในยีน *WNT10A* และการกลายพันธุ์แบบเฮเทอโรไซกัสที่มีการเปลี่ยนเบส c.413A> T (p.Asn138Ile) ในยีน *EDARADD* ด้วย ในผู้ป่วยรายที่สาม พบการกลายพันธุ์แบบเฮเทอโรไซกัสที่มีการเปลี่ยนเบส c.511C> T (p.Arg171Cys) ในยีน *WNT10A*

สรุปผลการศึกษา การศึกษานี้รายงานความชุกของภาวะฟันหายแต่กำเนิดในประชากรไทยคิดเป็น 9.23% โดยพบการหายของฟันจำนวนหนึ่งซี่มากที่สุดและที่ฟันกรามน้อยล่างซี่ที่สองบ่อยที่สุด อีกทั้งยังศึกษาพบการการเปลี่ยนแปลงทางพันธุกรรมแบบโฮโมไซกัสหรือเฮเทอโรไซกัสในยีน *WNT10A* ในผู้ป่วยฟันหายอย่างน้อย 3 ราย โดยการเปลี่ยนแปลงทางพันธุกรรม c.511C> T (p.Arg171Cys) และ c.916_918dupAAC (p.Asn306dup) ใน *WNT10A* นี้พบทั้งในผู้ที่มีฟันหายแต่กำเนิดและมีฟันครบทุกซี่ จึงเป็นไปได้ว่าการกลายพันธุ์มีการแสดงออกไม่สมบูรณ์ อย่างไรก็ตามการกลายพันธุ์ที่พบในการศึกษานี้จะเป็นสาเหตุที่แท้จริงของภาวะฟันหายหรือไม่ต้องอาศัยการศึกษาทางโมเลกุลและจำนวนตัวอย่างเพิ่มเติมในอนาคต

สาขาวิชา	ทันตกรรมผู้สูงอายุและการดูแลผู้ป่วยพิเศษ	ลายมือชื่อนิสิต .....
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		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORD: Genetics, Hypodontia, Mutations, Oligodontia, Panoramic radiography, Tooth agenesis
Charinya Kanchanasevee : TOOTH AGENESIS IN THAI POPULATION: PREVALENCE, CHARACTERISTICS AND ITS GENETIC VARIANT IN A THAI FAMILY. Advisor: Assoc. Prof. THANTRIRA PORNTAVEETUS, D.D.S., Ph.D Co-advisor: Asst. Prof. PISHA PITTAYAPAT, D.D.S., Ph.D

Objectives: To assess the prevalence and characteristics of tooth agenesis in Thai population and to identify the genetic variants in a family affected with tooth agenesis.

Methods: Panoramic radiographs in a random sample of Thai patients from 15 to 20 years of age which were taken in 2017 at the Faculty of Dentistry, Chulalongkorn University. Demographic data (age and sex) and dental characteristics of the patients were recorded. A chi-square test was used to determine the differences in the prevalence of tooth agenesis between sexes, maxillary-mandibular teeth, left-right sides, and numbers of missing teeth. Three mL of blood samples were collected for DNA extraction and subjected for mutation analyses.

Results: The overall prevalence of tooth agenesis, excluding the third molars, was 9.23% or 101 out of 1090 patients. The prevalence in females (9.95%) was higher than males (8.23%). According to tooth type, the most common missing tooth was the mandibular second premolars (25.00%; n=55), followed by mandibular lateral incisors (23.28%; n=51), and maxillary lateral incisors (15.91%; n=35). The single most common missing tooth was the mandibular right lateral incisor (15.00%; n=33), followed by the mandibular right second premolar (12.70%; n=28), mandibular left second premolar (12.27%; n=27), and maxillary right lateral incisor (8.64%, n=19). Of all the 101 patients with tooth agenesis, 43.56% had one missing tooth, 41.58% had two missing teeth, 4.95% had three to four missing teeth, and 5.94% had six or more missing teeth. By location, tooth agenesis was found more often on the right side (54.09%) than on the left side (45.91%), and more in mandibular arch (61.82%) than maxillary arch (38.18%). Mutations in *WNT10A* are the most frequently found in the unrelated 11 probands affected with tooth agenesis (27.27%, n= 3). In the 3 tooth agenesis individuals, the cases were 9 missing teeth, 8 missing teeth, and 2 missing teeth. The first proband was found to have the heterozygous duplication, c.916_918dupAAC (p.Asn306dup), in *WNT10A*. The second proband possessed the homozygous missense variant, c.511C>T (p.Arg171Cys), in *WNT10A* and the heterozygous missense variant, c.413A>T (p.Asn138Ile), in *EDARADD*. The third proband harbored the heterozygous missense variant, c.511C>T (p.Arg171Cys), in *WNT10A*.

Conclusions: This study reports the prevalence of congenital missing teeth at 9.23% in Thai population. A single tooth absence is most common and according to types of tooth, the mandibular second premolar is the most frequently missing tooth. We identify 3 probands affected with tooth agenesis who have either homozygous or

Field of Study:	Geriatric Dentistry and Special Patients Care	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....

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Charinya Kanchanasevee

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CHAPTER I

INTRODUCTION

Background and rationale

Tooth agenesis is the most common anomaly of the permanent dentition. Missing teeth can lead to several consequences including esthetic and mastication problems, dislocation of non-affected teeth in dental arch, alveolar bone atrophy, and malocclusion. Therefore, early intervention and appropriate treatment are vital (1).

The prevalence of tooth absence varies according to the studied population. The prevalence of 5.5% was reported in European; 3.9% in North American; and 6.4% in the Australian population. The highest prevalence, 6.9%, was found in an Asian population (2). Nonetheless, few epidemiological studies have been carried out in an Asian population. Up to date, the prevalence and pattern of tooth agenesis in Thailand was reported in only two studies. Tooth agenesis is frequently associated with other dental anomalies such as microdontia, retained primary teeth, delayed dental age, and can be a part of the syndrome (3). The knowledge of the pattern and prevalence of tooth agenesis is therefore important for dental treatment.

Genetic factors play a fundamental role in the etiology of hypodontia. Recent advance in human genetics has improved our understanding of the cause of tooth agenesis. Several genes have been identified including muscle segment homeobox 1 (*MSX1*), paired box 9 (*PAX9*), ectodysplasin A, and *WNT10A* as the causes of tooth agenesis (4). Identifying the genetic causes of tooth absence facilitates the diagnosis

and comprehensive treatment for the affected individuals and their family members. Therefore, this study aims to assess the prevalence and characteristics of tooth agenesis in Thai population and to identify the genetic mutations in affected patients. The findings will expand the clinical, epidemiological, and genetic knowledge of tooth agenesis.

Research questions

1. What is the prevalence of tooth agenesis in Thai population?
2. What are the characteristics of tooth agenesis in Thai population?
3. What are genetic variants identified in a family affected with tooth agenesis?

Research objectives

1. To assess the prevalence of tooth agenesis in Thai population
2. To assess the characteristics of tooth agenesis in Thai population
3. To identify the genetic variants in a family affected with tooth agenesis

Research hypothesis

1. Prevalence of tooth agenesis in Thai population is similar to that in other populations.
2. Characteristics of tooth agenesis in Thai population are similar to those in other populations.
3. Tooth agenesis is related to genetic mutation.

Scope of research

The aim of this study was to assess the prevalence and pattern of tooth agenesis in Thai dental patients and to identify the genetic variants in the patients

affected with tooth agenesis. The study design was a retrospective cross-sectional study of panoramic radiographs in a random sample of Thai patients from 15 to 20 years of age which were taken in 2017 at the Faculty of Dentistry, Chulalongkorn University. Demographic data (age and sex) and dental characteristics of the patients were recorded. A chi-square test was used to determine the differences in the prevalence of tooth agenesis between sexes, maxillary-mandibular teeth, left-right sides, and numbers of missing teeth. Three ml. of blood samples were collected for DNA extraction and subjected for mutation analyses. The informed consents were obtained from each participant. The researcher recorded the history and performed clinical, radiographic, and laboratory examinations of the patients.

Limitation

The samples were the patients attending at Faculty of Dentistry, Chulalongkorn University, who may not represent the overall Thai population.

Expected outcomes

1. Providing data of prevalence and characteristics of tooth agenesis in Thai population
2. Identification of genetic mutations related to tooth agenesis
3. Expanding the epidemiological, characteristic, and genetic knowledge of tooth agenesis.

Keywords

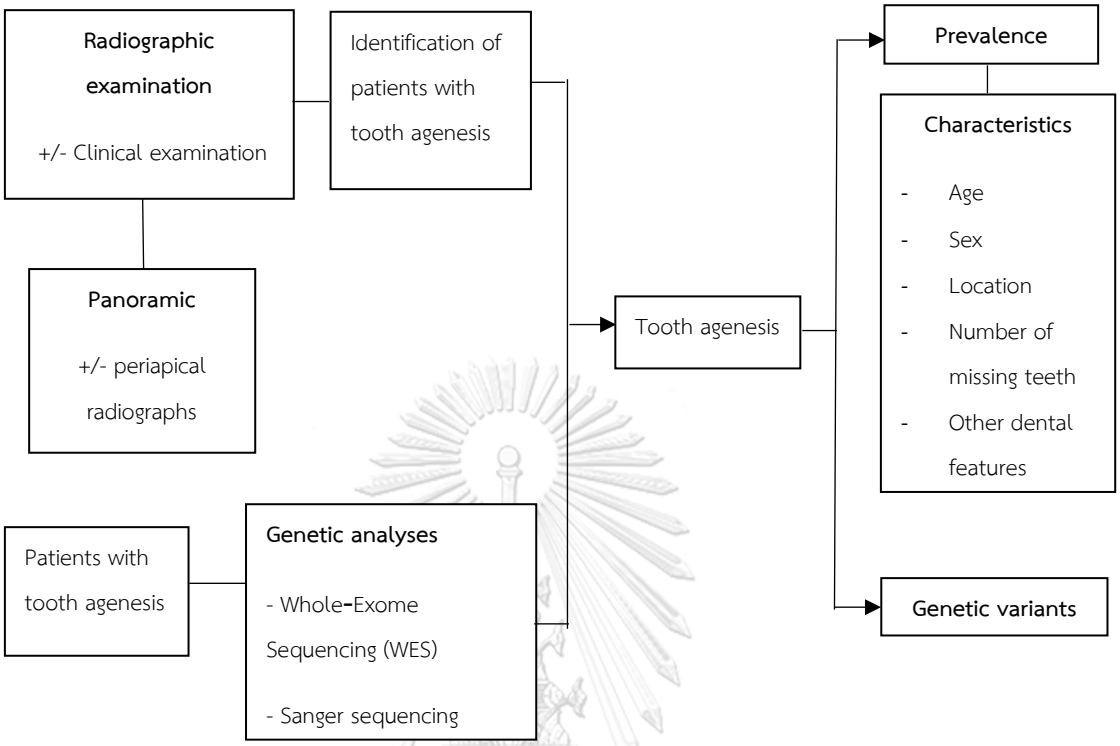
Genetics, Hypodontia, Mutations, Oligodontia, Panoramic radiography,

Tooth agenesis

Research design

Retrospective cross-sectional study and experimental research

Conceptual framework



CHAPTER II

LITERATURE REVIEW

Tooth agenesis, dental aplasia, or congenital missing teeth (CMT), is defined as developmental absence of at least one tooth. It is one of the most common developmental anomalies caused by the disturbances during the early stages of tooth development (5).

Hypodontia refers as an absence of less than six teeth. More than six missing teeth is defined as oligodontia. The complete absence of teeth is termed as anodontia. Tooth agenesis may occur as non-syndromic (isolated) or syndromic form which is associated with systemic anomalies including cleft lip, cleft palate, ectodermal dysplasia, Down syndrome, Rieger syndrome, and Book syndromes (6).

Features associated with tooth agenesis

Dental features: Microdontia is a widely reported finding associated with tooth agenesis. Delayed tooth development, abnormal tooth size and shape, molar taurodontism, ectopic tooth eruption, enamel hypoplasia, peg maxillary lateral incisors, primary molar infraocclusion, and palatally inclined or impacted maxillary canines have been observed in patients with tooth agenesis (3). Isolated hypodontia has been shown to impact the development of adjacent teeth resulting in the decreased crown size, altered crown and root morphology, delayed development, and taurodontism (7).

Skeletal features: Class III skeletal malocclusion (8), short and retroclined upper arch, and proclined upper incisors have been reported (9).

Systemic features: syndromic tooth agenesis could be related to other medical problems. For example, patients with kabuki syndrome have hypodontia, malocclusion, high-arched palate, heart diseases, and intellectual disability. Microdontia, pointed anterior teeth, and oligodontia are commonly present in ectodermal dysplasia patients who have anomalies of hairs, teeth, nails, and sweat glands (10).

Prevalence of tooth agenesis

Previous studies showed variations in the prevalence of tooth agenesis among populations in different ethnic backgrounds (11, 12). In the primary dentition, the frequency was between 0.1% and 2.4% (6). The primary dental aplasia was usually followed by permanent tooth missing. The prevalence of agenesis of the permanent teeth excluding the third molars ranged between 0.15% and 16.2% which was observed in studies varying in population size from 200 to over 100,000 subjects (6). In Thailand, two prevalence studies of hypodontia in orthodontic patients have reported different results. The prevalence of hypodontia reported by Weeraya *et al.* (2015) was 13.7% (84 out 638 patients in upper central area of Thailand) which was lower than that reported by Kositbowornchai *et al.* (2010) at 26.4% (150 out 570 patients in northeastern area of Thailand) (13, 14).

Location of tooth absence

Majorities of studies reported the similar occurrence of tooth agenesis in the maxilla and the mandible (14, 15). Third molars were the most commonly absent teeth in the dentition. When the third molar was excluded from studies, the reported prevalence rates for each tooth varied according to the population (4). Polder *et al.* (2004) found that absence of maxillary lateral incisors usually occurred bilaterally whereas unilateral agenesis was commonly found in the second mandibular premolar (15). In Thai population, Weeraya *et al.* (2015) showed that the most commonly missing tooth was the lower lateral incisor (26.32%), followed by lower premolars (24.81%) and upper lateral incisor (19.55%)(13). Kositbowornchai *et al.* (2010) observed that the lower lateral incisor had the highest prevalence of absence, followed by the upper lateral incisor and lower second premolar respectively (14).

Sex

No significant differences in sex was related to the missing of primary teeth (16). In contrast, females were more affected by hypodontia than males in the permanent dentition. One meta-analysis found the incidence of tooth agenesis in females was 1.4 time higher than that in males (15).

Ethnicity

Previous studies showed a prevalence of hypodontia at 5.5% in European, 3.9% in North American, 6.4% in Australian (15), and 6.9% in an Asian population (4). The highest prevalence was found in the Chinese population (7.7% in women and 6.1% in

men). In contrast, the lowest prevalence rate of 2.2% was found in the Saudi Arabian women.

Dental age

Several studies indicated a delayed dental age in children with hypodontia (17, 18). Jozo Badrov *et al.* (2017) used the method of Haavikko (1970) to determine the developmental stages of all developing permanent teeth on 345 panoramic radiographs. The difference between dental age and chronological age was calculated in hypodontia group, compared with non-hypodontia group. The result showed that dental age was significantly delayed in tooth agenesis patients. The mean differences were −0.57 ± 1.20 years and −0.61 ± 1.23 years in males and females ($p < 0.001$), without difference between sexes ($p = 0.763$) (19). The second lower premolars, first lower premolar, and second lower molars showed pronounced developmental delay (17, 19).

Etiology

Tooth agenesis is related to numerous etiologies including genetic and environmental factors. Environmental factors include medications, low birth weight, malnutrition, vitamin D deficiency, infections, and metabolic disorders (2).

Previous studies demonstrated a strong genetic influence on hypodontia. Monozygotic twin and familial studies determined that agenesis of lateral incisors and premolars was inherited as autosomal dominant trait with incomplete penetrance and

variable expressivity. Furthermore, it was suggested that anterior tooth agenesis was likely to be related to genes while missing posterior teeth might be sporadic (20).

Tooth agenesis has been associated with mutations in several genes including muscle segment homeobox 1 (*MSX1),* paired box gene 9 (*PAX9),* axis inhibition protein 2 *(AXIN2),* ectodysplasin A *(4),* sprouty RTK signaling antagonist 2 *(SPRY2),* transforming growth factor alpha *(TGFA),* sprouty RTK signaling antagonist 4 *(SPRY4),* Wnt family member 10A *(WNT10A),* fibroblast growth factor 3 *(FGF3),* fibroblast growth factor 10 (*FGF10),* fibroblast growth factor receptor 2 (*FGFR2), and* bone morphogenetic protein 4 *(BMP4).* Among these genes, *MSX1, PAX9*, *AXIN2*, *WNT10A* and *EDA* are the most frequently reported genes associated with non-syndromic tooth agenesis These genes play roles in signaling pathways related to tooth development (Table 1) (21).

Table 1 Genes involved in isolated tooth agenesis

Gene	OMIM	Chromosome	Dental Phenotypes
AXIN2	604025	17q24.1	Oligodontia, hypodontia
ANTXR1	606410	2p13.3	Oligodontia, hypodontia
COL17A1	113811	10q25.1	Hypodontia
DKK1	605189	10q21.1	Hypodontia
EDA	300451	Xq13.1	Oligodontia, hypodontia
EDAR	604095	2q13	Oligodontia, hypodontia
EDARADD	606603	1q42-q43	Oligodontia, hypodontia
FGFR1	136350	8p11.23	Hypodontia
GREM2	608832	1q43	Hypodontia, microdontia, taurodontia
IRF6	607199	1q32.2	Hypodontia, lip pits
MSX1	142983	4p16.2	Oligodontia, hypodontia
LAMA3	600805	18q11.2	Hypodontia
LRP6	603507	12p13.2	Oligodontia
LTBP3	602090	11q13.1	Oligodontia, hypodontia
PAX9	167416	14q13.3	Oligodontia, hypodontia, microdontia
SMOC2	607223	6q27	Oligodontia, microdontia, abnormal morphology
WNT10A	606268	2q35	Oligodontia, hypodontia
WNT10B	601906	12q13.12	Oligodontia, microdontia

MSX1 is a member of the homeobox genes expressed in regions of condensing ectomesenchyme in the tooth germ. *MSX1* mutations are the first to be described in individuals associated with non-syndromic tooth agenesis. Most of which are nonsense or missense mutations located in the homeobox domain. Maxillary and mandibular second premolars and maxillary first premolars are the most common missing teeth (21).

PAX9 is a transcription factor expressed in tooth mesenchyme during tooth morphogenesis. Mutations in *PAX9* are implicated in arrested tooth development at

the bud stage. Heterozygous mutations in *PAX9* are associated with non-syndromic hypodontia. *PAX9* variants are associated with a high risk of agenesis of the permanent second molars, followed by second premolars; a few reports of agenesis of anterior teeth are also exist. In general, the severity of the tooth agenesis phenotype is associated with the type of mutation and its impact on PAX9 function. Individuals with nonsense/frameshift mutations present with more severe phenotype when compared to those with missense mutations. Smaller crown dimensions throughout the dentition have also been reported in tooth agenesis patients with *PAX9* mutations (21).

AXIN2 is involved in cell growth, proliferation, and differentiation. Its mutations are associated with agenesis of molars, lower incisors and upper lateral incisors. The absence of at least one incisor is frequently reported. Five *AXIN2* mutations have been widely reported in the literature, including four missense (c.956+16A>G; p.Pro50Ser, c.2051C>T; p.Ala684Val, c.2062C>T; p.Leu688Leu, and c.2272G>A; p.Ala758Thr), and one frameshift (c.1994insG; p.Asn666GlyfsX41). The presence of this frameshift mutation is associated with more missing teeth than missense mutations in all affected individuals (21).

EDA is found to be involved in X-linked hypo-hidrotic ectodermal dysplasia (HED). Several studies have reported sporadic hypodontia in families affected by mutations in *EDA* and *EDA* receptor (*EDAR*) genes. *EDA* mutations have also been involved in the missing of maxillary lateral incisors (22).

Among the large family of WNT members, *WNT10A* seems to have a role in tooth formation during odontoblast differentiation. *WNT10A* mutations have been associated with various ectodermal dysplasia syndromes, ranging from severe autosomal recessive Schopf-Schulz-Passarge syndrome (SSPS) to odonto-onycho-dermal dysplasia (OODD) and autosomal dominant missing teeth (23). OODD and SSPS share common characteristics, including severe tooth agenesis.

Recent studies have found that mutations in *WNT10A* are also associated with non- syndromic hypodontia. For example, Kantaputra and Sripathomsawat (2011) reported that a mutation in this gene gave rise to missing teeth in an American family (23). Van den Boogaard *et al.* (2012) suggested that such mutations were present in more than half of non-syndromic hypodontia cases (24). Variants in *WNT10A* were identified in 15.8% of tooth agenesis patients with 1 to 3 missing teeth, and in 50% of patients with more than 4 missing teeth. Of note, the heterozygous *WNT10A* variants were identified in unaffected individuals in tooth agenesis families, as well as in unrelated control individuals with no tooth agenesis or family history of tooth agenesis. It was estimated that approximately 41% of individuals showing a single heterozygous variant in *WNT10A* did not have tooth agenesis (21).

Furthermore, the study of Kantaputra *et al.* (2011) in Chiangmai Province (Northern part of Thailand) demonstrated that agenesis of the maxillary permanent canines was a distinct entity, associated with mutations in *WNT10A* (23). Inheritance

appeared to be autosomal dominant. It was also proposed that agenesis of the maxillary permanent canines may accompany by microdontia of the maxillary permanent lateral incisors and dens invaginatus of the maxillary permanent lateral incisors (25).

Recently, variants of the *WNT10A* gene were described to be present in almost 50% of all Caucasian cases with non-syndromic tooth agenesis. Furthermore, in the Chinese population, *WNT10A* variant frequencies were shown to be significantly increased in patients with non-syndromic tooth agenesis compared with individuals with full dentition. An investigation of 50 Japanese patients with severe congenital tooth agenesis identified 11 patients with *WNT10A* variants (26).

A few *WNT10A* variants were suggested to be common 'hotspots' for mutations in specific populations. For example, the c.637G>A (p.Gly213Ser) variant was found more frequently in Asian populations, meanwhile the c.682T>A (p.Phe228Ile) variant was widely reported in homozygous or heterozygous forms in Caucasian individuals with tooth agenesis, but also in normal controls at a frequency of 2.3%. The Phe228Ile variant was the most commonly found *WNT10A* variant, and often described in combination with additional variants in *WNT10A* or in other genes (Figure 1) (21).

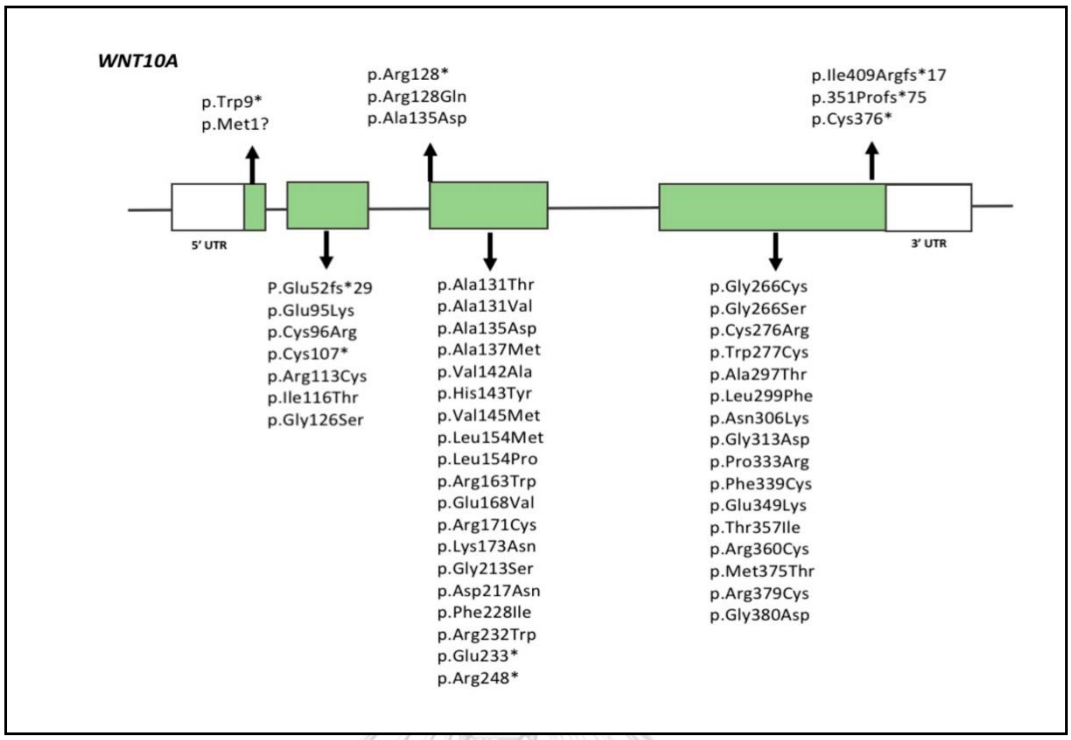


Figure 1 Location of reported missense, frameshift, and nonsense mutations in WNT10A genes. Green boxes represent exons. Horizontal lines between exons represent introns. UTR: Untranslated region.

Although there are several reports of hypodontia in many countries, however, the understanding of tooth agenesis in Thai population is still limited. In addition, the knowledge about genetic variants associated with tooth missing has not been well understood. This study therefore aims to investigate the prevalence, characteristics, and genetic mutation associated with tooth absence in Thai population. Our findings have expanded the knowledge in tooth agenesis, one of the most common dental anomalies, in both clinical and genetic aspects.

CHAPTER III

RESEARCH METHODOLOGY

Subject enrollment

The sample size was calculated from n4Studies. The Proportion (p) = 0.13, Error (d) = 0.02, Alpha (**α**) = 0.05, and Z (0.975) = 1.959964 were substituted in the following formula. In this study, the sample size (n) obtained was 1087.

$$n = \frac{z^2_{1-\frac{\alpha}{2}} p(1-p)}{d^2}$$

Figure 2 The sample size formula

1,200 panoramic radiographs of Thai dental patients taken between 1 January 2017 and 31 December 2017 at the Faculty of Dentistry, Chulalongkorn University were retrospectively collected. The inclusion criteria were the individual with Thai nationality and 15 – 20 years of age on the day the radiographs were taken. The radiographs with a doubtful diagnosis, for example, the teeth that might be loss due to trauma or previous extraction, were excluded. In cases with unclear diagnosis, previous dental history, dental casts, or any available information were examined. Subjects with congenital anomalies, records of extraction of the permanent teeth, trauma, and prior orthodontic treatment were excluded. By exclusion of 110 radiographs, the final samples of this study included 1,090 panoramic radiographs.

Ethical consideration

The research protocol was approved by the Human Research Ethic Committee of Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2018-091). All participants were informed about the research information and written informed consent was obtained.

Image observation

All selected radiographs were examined by the same operator to identify the presence of dental agenesis (excluding third molars). A tooth was diagnosed as congenitally missing if crown mineralization could not be identified on panoramic radiographs.

The panoramic radiographs were taken by CS8000c, CS9000c radiographic units (Carestream Health., Inc., Rochester, USA) and Veraviewepocs 3D (J. Morita, Kyoto, Japan). Standard radiographic parameters were set according to patients' sizes. All panoramic images were stored in the hospital picture archiving and communication system (PACS).

By using Infinitt® PACS software (Infinitt Healthcare Co., Ltd., Seoul, South Korea), the database was searched for panoramic radiographs that fit the criteria (age 15 to 20 years old, radiographic date between 1 January 2017 and 31 December 2017). The observer was also allowed to use the PACS software tools such as window/level and zoom.

The diagnosis of tooth agenesis

The diagnosis of tooth agenesis was based on the teeth that failed to erupt in the oral cavity and absence of crown mineralization on the radiographs. A tooth was diagnosed as missing when it could not be identified or discerned radiographically without any evidence of extraction. If a definite diagnosis of hypodontia cannot be made, the particular radiographs were excluded. The data describing the pattern of tooth absence are age, sex, location (right or left sides, anterior or posterior regions, maxilla or mandible teeth) and number of missing teeth was recorded systematically. Tooth number used to represent each tooth was according to Federation Dentaire International (FDI) (27). The obtained data was recorded by Microsoft Excel 2010.

Mutation analysis

Eleven unrelated Thai probands (an individual affected with tooth agenesis) and their family members were recruited for the mutation analysis with written informed consents. Clinical and radiographic examinations were performed at Faculty of Dentistry, Chulalongkorn University. Photographs and panoramic and/or periapical radiographs were taken. Blood samples from the probands and their family members were collected. A pedigree of each family was constructed by extended interviews.

We collected a family in which tooth agenesis was segregating in an autosomal-dominant manner to define the clinical features of hypodontia and to localize the gene locus behind this anomaly. Retrospective data were reviewed and the diagnosis of tooth agenesis was verified by panoramic dental radiographs for all available family

members. Three members of each family were studied, with one member being affected and the parents unaffected.

The patient's genomic DNA was extracted from peripheral blood leukocytes and sent to Macrogen Inc. (Seoul, Korea) for next-generation sequencing (NGS). DNA was captured on the TruSeq Exome Enrichment Kit (Illumina) and subsequently sequenced on the Hiseq2000 Instrument. The raw data per exome was mapped to the human reference genome hg19 using CASAVA v1.7. Variant calling was performed using SAM tools (http://samtools.sourceforge.net/). The sequencing data was mapped to NCBI37 reference human genome (the version used for 1000 Genomes project, Exome Variant Server (EVS), and 2,166 in-house database (THWES2166). The variants were validated by Sanger sequencing.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM, Armonk, NY). The significant difference among groups was determined by the Chi-square test. The level of significance was set at $P < 0.05$. The statistical analysis was used to compare the prevalence of tooth agenesis between maxillary-mandibular arches, right-left sides, and males and females.

CHAPTER IV

RESULTS

Clinical and radiographic examinations

A total of 1,090 panoramic radiographs of healthy patients aged 15-20 years, 643 females and 447 males were examined. Tooth agenesis in the permanent dentition (excluding third molars) was diagnosed in 101 subjects. The overall prevalence of tooth agenesis was found to be 9.23%. The prevalence of tooth agenesis in females was higher than males (9.95 % and 8.23 %, respectively) (Table 2).

Table 2 Distribution of prevalence of tooth agenesis by sex

Sex	Number of patients		Prevalence (%)
	Examined	Affected	
Male	447	37	8.23
Female	643	64	9.95
Total	1090	101	9.23

The most common single congenitally missing teeth were the mandibular right lateral incisors (15.00%; n = 33), followed by the mandibular right second premolar (12.73%; n = 28), the mandibular left second premolar (12.27%; n = 27), the maxillary right lateral incisors (8.64%, n = 19), the mandibular left lateral incisors (8.18%; n = 18), the maxillary left lateral incisors (7.27%; n = 16), the maxillary left second premolars (5.45%; n = 12), the maxillary right and left first premolars and the maxillary right second premolars (4.55%; n = 10) respectively. In this study, tooth agenesis was not found in the maxillary right central incisors, mandibular left canines, mandibular first molars and all second molars. (Table 3).

Table 3 Distribution of prevalence of tooth agenesis by tooth (n = 220)

Tooth	Sex (n)		Total (%)	Sig. (P < 0.05)
	Male (%)	Female (%)		
17	0	0	0 (0)	1.000
16	0	0	0 (0)	1.000
15	5	5	10 (4.55)	0.508
14	3	7	10 (4.55)	0.746
13	1	2	3 (1.36)	1.000
12	8	11	19 (8.64)	0.805
11	0	0	0 (0)	1.000
21	1	1	2 (0.91)	1.000
22	10	6	16 (7.27)	0.057
23	0	1	1 (0.45)	1.000
24	3	7	10 (4.55)	0.746
25	5	7	12 (5.45)	0.768
26	1	0	1 (0.45)	1.000
27	0	0	0 (0)	1.000
31	3	2	5 (2.27)	0.299
32	5	13	18 (8.18)	0.452
33	0	0	0 (0)	1.000
34	2	7	9 (4.09)	0.489
35	11	16	27 (12.27)	0.833
36	0	0	0 (0)	1.000
37	0	0	0 (0)	1.000
41	3	4	7 (3.18)	0.776
42	7	26	33 (15)	0.05
43	1	2	3 (1.36)	1.000
44	3	3	6 (2.73)	0.675
45	11	17	28 (12.73)	0.838
46	0	0	0 (0)	1.000
47	0	0	0 (0)	1.000
Total	**83 (37.73)**	**137 (62.27)**	**220 (100)**	

The number of missing teeth per patient ranged from 1 to 14. All of 101 patients with tooth agenesis, 43.56% had one missing tooth, 41.58% had two missing teeth, 8.91% had three to four missing teeth, and 5.94% had six or more missing teeth

(oligodontia). The differences in the prevalence according to number of missing teeth between sexes were not statistically significant (Table 4).

Table 4 Distribution of prevalence of tooth agenesis by numbers of missing teeth (n = 101)

Number of missing teeth	Sex		Total (%)	Sig (P<0.05)
	Male (%)	Female (%)		
1	21 (20.79)	23 (22.77)	44 (43.56)	0.097
2	11 (10.89)	31 (30.69)	42 (41.58)	0.061
3	2 (1.98)	3 (2.97)	5 (4.95)	1.000
4	0 (0)	4 (3.96)	4 (3.96)	0.294
5	0 (0)	0 (0)	0 (0)	1.000
≥6	4 (3.96)	2 (1.98)	6 (5.94)	0.194
Total	**38 (37.62)**	**63 (62.38)**	**101 (100)**	

Distribution and statistical comparisons of an absence of the same tooth type in the right or left side in relation to the dental arches are shown in Table 5. Statistically significant difference was found for the comparison between teeth 14 and 44.

Table 5 Frequency of tooth agenesis in relation to the maxillary and mandibular arches

Maxillary arch		Mandibular arch			Maxillary arch		Mandibular arch		
Tooth Number	Number (n)	Tooth Number	Number (n)	Sig (P<0.05)	Tooth Number	Number (n)	Tooth Number	Number (n)	Sig (P<0.05)
11	0	**41**	7	0.051	**21**	2	**31**	5	0.696
12	19	**42**	33	0.848	**22**	16	**32**	18	0.522
13	3	**43**	3	0.664	**23**	1	**33**	0	0.416
14	10	**44**	6	0.023*	**24**	10	**34**	9	0.310
15	10	**45**	28	0.217	**25**	12	**35**	27	0.099
16	0	**46**	0	1.000	**26**	1	**36**	0	0.234
17	0	**47**	0	1.000	**27**	0	**37**	0	1.000

* P-value <0.05

Frequency of tooth absence in the maxilla or mandible in relation to right and left side are shown in Table 6. No statistical differences were detected (Table 6).

Table 6 Frequency of tooth agenesis in relation to the right and left sides in the same dental arch

Maxillary arch					Mandibular arch				
Right side		Left Side			Right Side		Left Side		
Tooth Number	Number (n)	Tooth Number	Number (n)	Sig (P<0.05)	Tooth Number	Number (n)	Tooth Number	Number (n)	Sig (P<0.05)
11	0	**21**	2	0.494	**41**	7	**31**	5	1.000
12	19	**22**	16	0.658	**42**	33	**32**	18	0.156
13	3	**23**	1	0.616	**43**	3	**33**	0	0.125
14	10	**24**	10	1.000	**44**	6	**34**	9	0.169
15	10	**25**	12	0.804	**45**	28	**35**	27	0.294
16	0	**26**	1	1.000	**46**	0	**36**	0	1.000
17	0	**27**	0	1.000	**47**	0	**37**	0	1.000

With regards to the maxillary or mandibular dental arch, tooth agenesis was found more in the mandibular arch (61.82%, n = 136) than the maxillary arch (38.18%, n = 84). Statistically significant differences were found for first and second premolars (Table 7).

Table 7 Distribution of prevalence of tooth agenesis by tooth type in relation to maxillary-mandibular arches

Tooth	Maxillary arch (%)	Mandibular arch (%)	Total (%)	Sig (P<0.05)
Central incisor	2 (0.91)	12 (5.45)	14 (6.36)	0.057
Lateral incisor	35 (17.33)	51 (23.18)	86 (39.09)	0.538
Canine	4 (1.82)	3 (1.36)	7 (3.18)	0.304
First premolar	20 (9.09)	15 (6.82)	35 (15.91)	0.012*
Second premolar	22 (10.00)	55 (25.00)	77 (35.00)	0.031*
First molar	1 (0.45)	0 (0)	1 (0.45)	0.382
Second molar	0 (0)	0 (0)	0 (0)	1.000
Total	**84 (38.18)**	**136 (61.82)**	**220 (100)**	

* P-value <0.05

Tooth agenesis was found more often on the right side (54.09%; n = 119) than on the left side (45.91%; n = 101), but no statistically significant differences were detected (Table 8).

Table 8 Distribution of prevalence of tooth agenesis by tooth type in relation to left-right sides

Tooth	Right side (%)	Left side (%)	Total (%)	Sig (P < 0.05)
Central incisor	7 (2.06)	7 (2.06)	14 (4.12)	0.788
Lateral incisor	52 (23.63)	34 (15.45)	86 (39.09)	0.165
Canine	6 (2.73)	1 (0.45)	7 (3.18)	0.128
First premolar	16 (7.27)	19 (8.64)	35 (15.91)	0.355
Second premolar	38 (17.27)	39 (17.73)	77 (35.00)	0.323
First molar	0 (0)	1 (0.45)	1 (0.45)	0.459
Second molar	0 (0)	0 (0)	0 (0)	1.000
Total	**119 (54.09)**	**101 (45.91)**	**220 (100)**	

Mutational analysis

Eleven patients were recruited for genetic study. Characteristics of tooth missing in the patients were shown in Table 9. The mutations were identified in three out of eleven Thai probands.

Table 9 Mutational analysis of eleven isolated tooth agenesis cases in this study (2017-2018)

Patient	Sex	Type of variant	Gene	DNA change	Amino acid change	Novel/ known	Number of missing teeth	Tooth Number of missing teeth
1	Female	Unidentified	-	-	-	-	6	13, 23, 32, 34, 38, 42
2	Male	Unidentified	-	-	-	-	12	12, 14, 15, 24, 25, 32, 35, 37, 41, 42, 45, 47
3	Female	Unidentified	-	-	-	-	10	13, 14, 15, 23, 24, 25, 34, 35, 44, 45
4	Male	Unidentified	-	-	-	-	16	12, 14, 15, 18, 24, 25, 28, 32, 35, 37, 38, 41, 42, 45, 47, 48
5	Female	Unidentified	-	-	-	-	10	12, 13, 14, 15, 22, 23, 24, 35, 44, 45
6	Female	Duplication	Wnt10A	Het c.916_918dupAAC	p.Asn306dup	Novel	9	14, 15, 24, 25, 34, 35, 44, 45, 46
7	Female	Unidentified	-	-	-	-	15	12, 13, 14, 15, 18, 22, 23, 24, 25, 28, 35, 38, 44, 45, 48
8	Female	Unidentified	-	-	-	-	11	15, 14,13, 12, 22, 23, 24, 25, 35, 44, 45
9	Male	Missense/ SNV Missense/ SNV	EDARADD Wnt10A	Het c.413A>T Homo c.511C>T	p.Asn138Ile p.Arg171Cys	Novel Known	8	12, 14, 18, 22, 28, 34, 38, 48
10	Female	Unidentified	-	-	-	-	3	32, 42, 45
11	Female	Missense/ SNV	Wnt10A	Het c.511C>T	p.Arg171Cys	Known	2	35, 45 (12, 22 peg shaped)

Het, heterozygous; Homo, homozygous

Proband 6

The first proband, proband number 6, was a 16-year-old Thai girl. She had a missing of 9 permanent teeth, including all 8 premolars and lower right first molar. The permanent lower left second molar was extracted due to pulp necrosis. The mandibular edentulous ridge was hypoplastic. The teeth were widely spaced. She had anterior deep bite and malocclusion. Her maxillo-mandibular relationship is Skeletal class I (Skeletal class II tendency) with a retrognathic maxilla and orthognathic mandible (Figure 3). Her mother and grandmother also have tooth missing. Her aunt (II-5) has 32 permanent teeth.

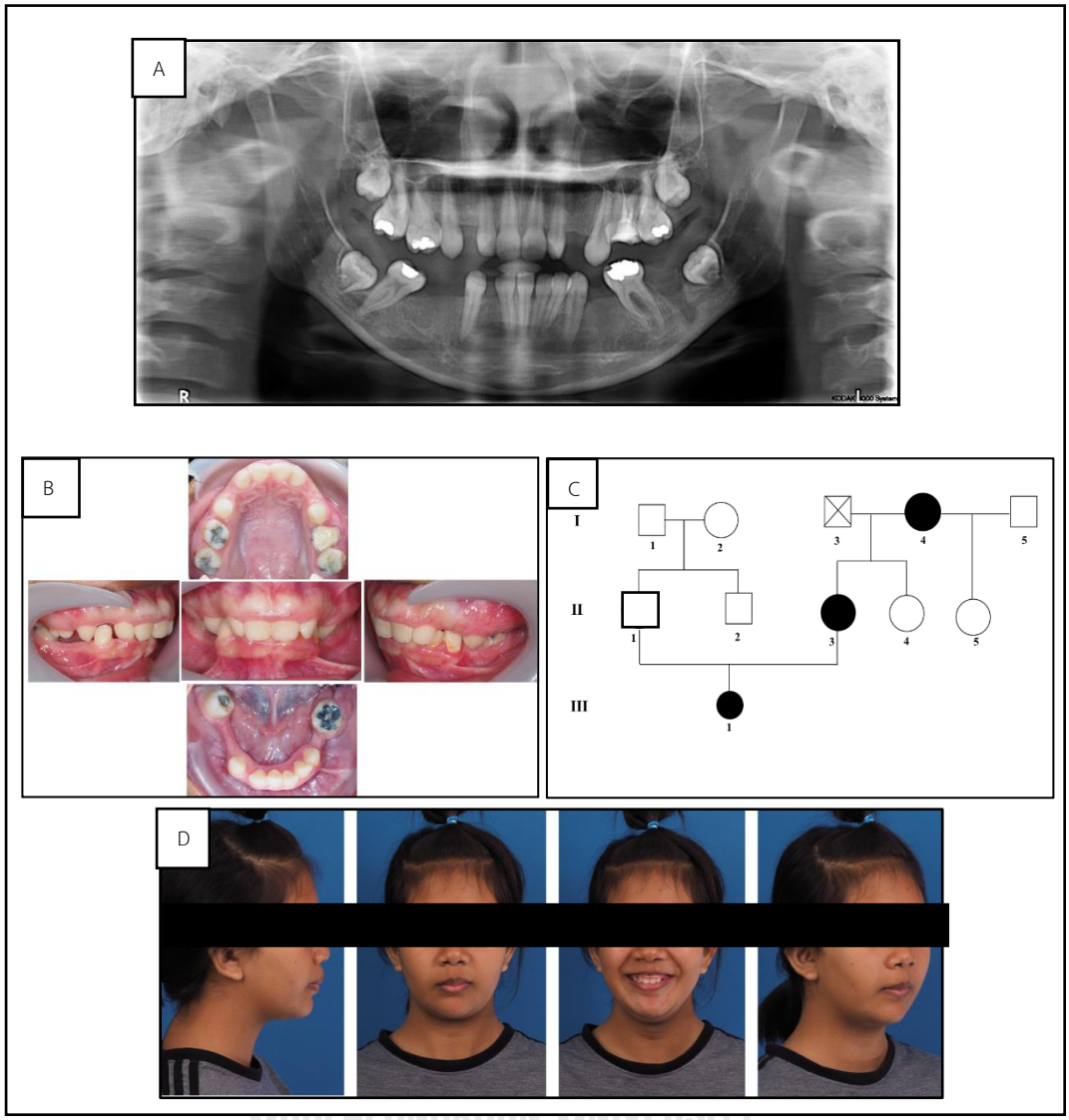


Figure 3 Clinical and radiographic features and pedigree of proband 6.

Panoramic radiograph shows multiple tooth missing (A). Intraoral photos and clinical features (B, D). Pedigree of the proband 6; males are identified by squares and females by circles. Filled symbols are marked as affected individuals and clear symbols identified as unaffected individuals. The proband is identified by the arrow (C).

A variant in the coding region of *WNT10A* was detected. The proband harbored the heterozygous duplication, c.916_918dupAAC, p.Asn306dup, in *WNT10A.* This variant was also found in her mother and grandmother who affected with tooth agenesis. This variant was also found in her unaffected aunt (II-5) (Figure 3 and Table 10).

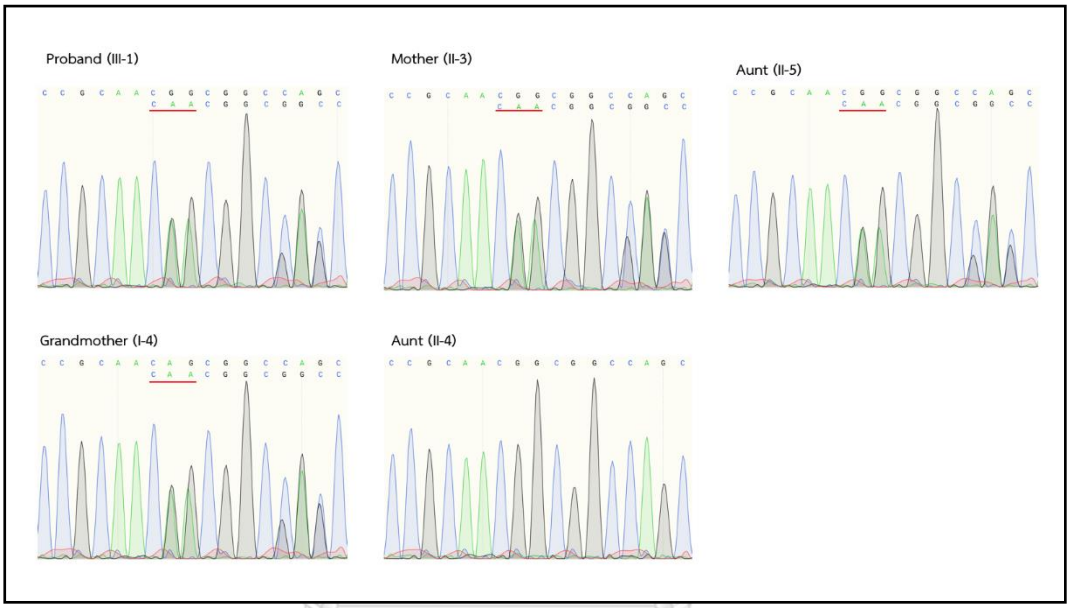


Figure 4 Chromatogram of proband 6's family

Chromatogram of proband 6's family shows the heterozygous duplication, c.916_918dupAAC, p.Asn306dup, in WNT10A.

Table 10 Mutational analysis and characteristics of tooth agenesis of proband 6

Characteristic	Family of Proband 6				
	Proband	**Mother**	**Grandmother**	**Aunt (II-5)**	**Aunt (II-4**
Sex	Female	Female	Female	Female	-
Missing teeth	Hypodontia	Hypodontia	Hypodontia	Normal	-
Peg-shaped teeth	None	None	None	None	-
Analysis method	Singleton	Sanger	Sanger	Sanger	-
Gene	*WNT10A*	*WNT10A*	*WNT10A*	*WNT10A*	-
WNT10A varient	c.916_918dupAAC	c.916_918dupAAC	c.916_918dupAAC	c.916_918dupAAC	-
Protien Change	p.Asn306dup	p.Asn306dup	p.Asn306dup	p.Asn306dup	-
Zygosity	Heterozygous	Heterozygous	Heterozygous	Heterozygous	-
ThWES	0	0	0	0	-

Proband 9

The second proband, proband 9, was a 34-year-old male. He had an absence of 8 permanent teeth including 2 upper lateral incisors, upper right first premolar, lower left premolar, and 4 third molars. He had the implants to replace the missing upper right lateral incisor and lower right second premolar (Figure 5. His sister (II-3) also affected with permanent tooth agenesis including 2 upper lateral incisors and 2 lower third molars.

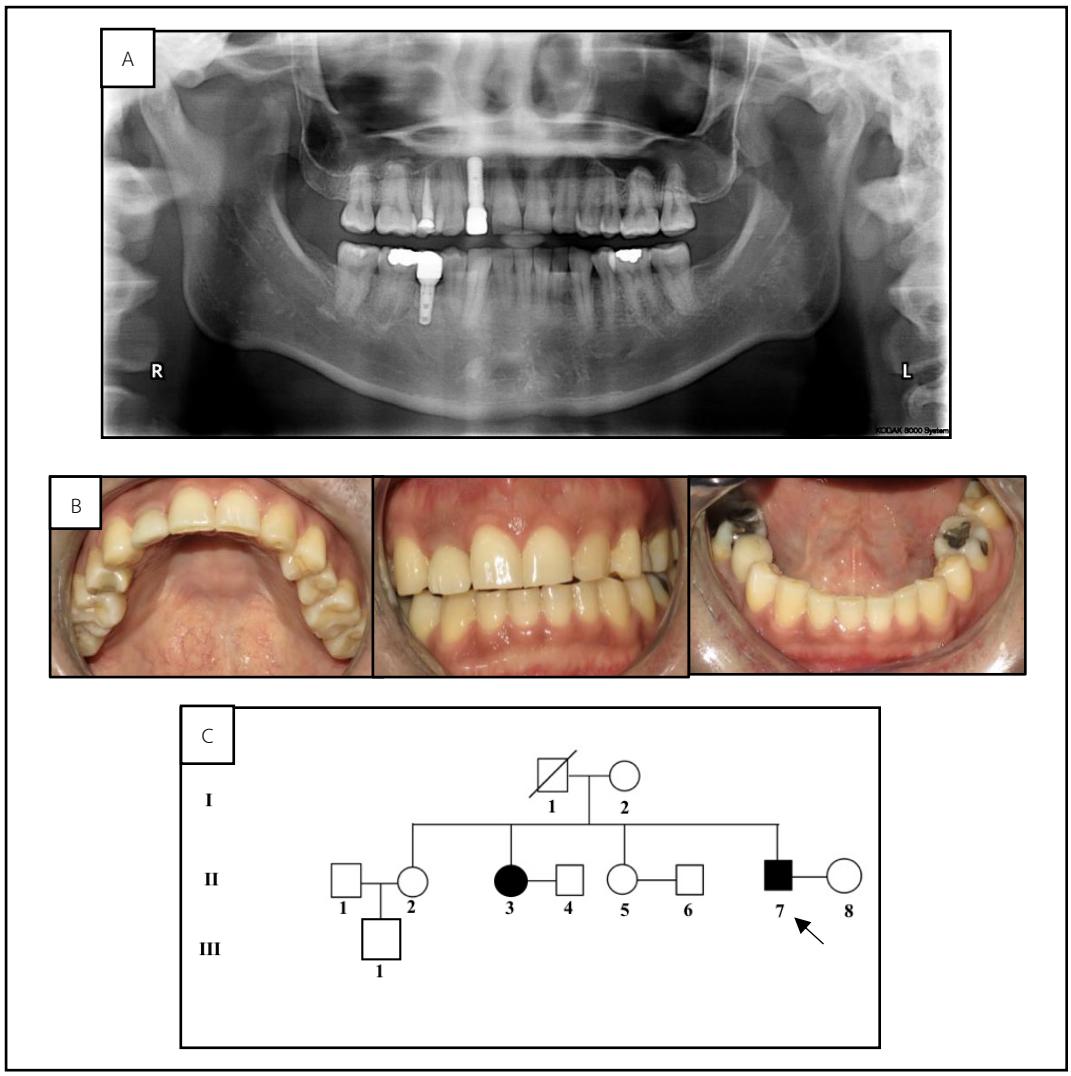


Figure 5 Clinical and radiographic features and pedigree of proband 9.

Panoramic radiograph shows multiple tooth missing (A). Intraoral photos (B). Pedigree of proband 9; males are identified by squares and females by circles. Filled symbols are marked as affected individuals and clear symbols identified as unaffected individuals. The proband is identified by the arrow (C).

The variants in the coding region of the *WNT10A* and *EDARADD* genes were detected in the proband. He possessed the homozygous missense variant, c.511C>T (p.Arg171Cys) in *WNT10A* and the heterozygous missense variant, c.413A>T (p.Asn138Ile) in *EDARADD* (Figure 6, 7). In addition, the heterozygous missense variant,

c.511C>T (p.Arg171Cys) in *WNT10A* was identified in his sister who had missing of upper right and left lateral incisors and his mother who did not have tooth missing but had peg-shaped upper lateral incisors (Figure 6 and Table 11).

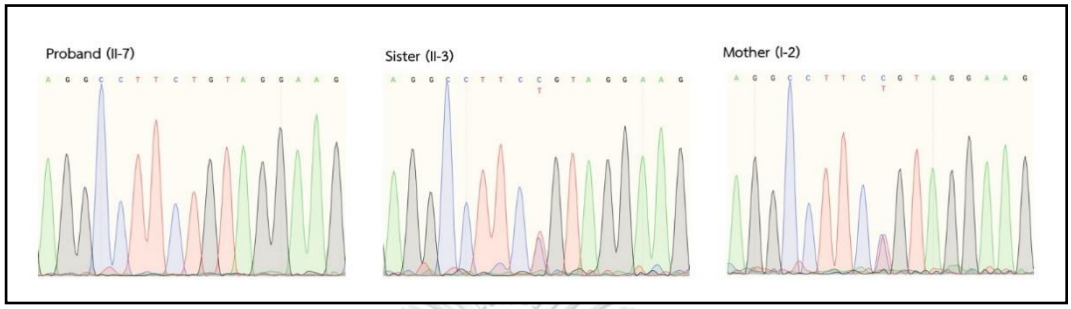


Figure 6 Chromatogram of WNT10a of proband 9's family shows the homozygous missense mutation, c.511C>T, p.Arg171Cys in WNT10A in the proband (II-7), a heterozygous missense mutation, c.511C>T, p.Arg171Cys in WNT10A in his sister (II-3) and his mother (I-2).

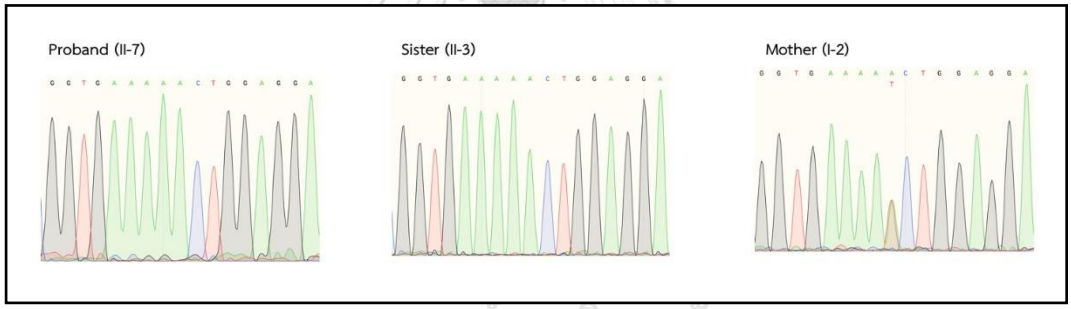


Figure 7 Chromatogram of proband 9 Chromatogram of proband 9 shows the heterozygous duplication, c.413A>T, p.Asn138Ile, in EDARADD.

Table 11 Mutational analysis and characteristics of tooth agenesis of proband 9

Characteristic	Family of Proband 9					
	Proband		Sister		Mother	
Sex	Female		Female		Female	
Missing tooth	Hypodontia		Hypodontia		Normal	
Peg-shaped teeth	No		No		Yes	
Analysis method	Singleton		Sanger		Sanger	
Gene	WNT10A	EDARADD	WNT10A	EDARADD	WNT10A	EDARADD
DNA change	c.511C>T	c.413A>T	c.511C>T	-	c.916_918dupAAC	-
Protien Change	p.Arg171Cys	p.Asn138Ile	p.Arg171Cys	-	p.Asn306dup	-
Zygosity	Homozygous	Heterozygous	Heterozygous	-	Heterozygous	-
ThWES	69	0	69	-	69	-

Proband 11

The third proband, proband 11, was a 31 year-old female with 2 missing permanent teeth, including lower right and lower left second premolars. Panoramic radiograph showed prolonged retention of deciduous lower right and left second molars and peg-shaped upper right and left lateral incisors. All third molars were extracted. Her parents did not have tooth missing. She reported that her unaffected sister (III-5) and niece (III-3) also have peg-shaped upper right and left lateral incisors (Figure 8).

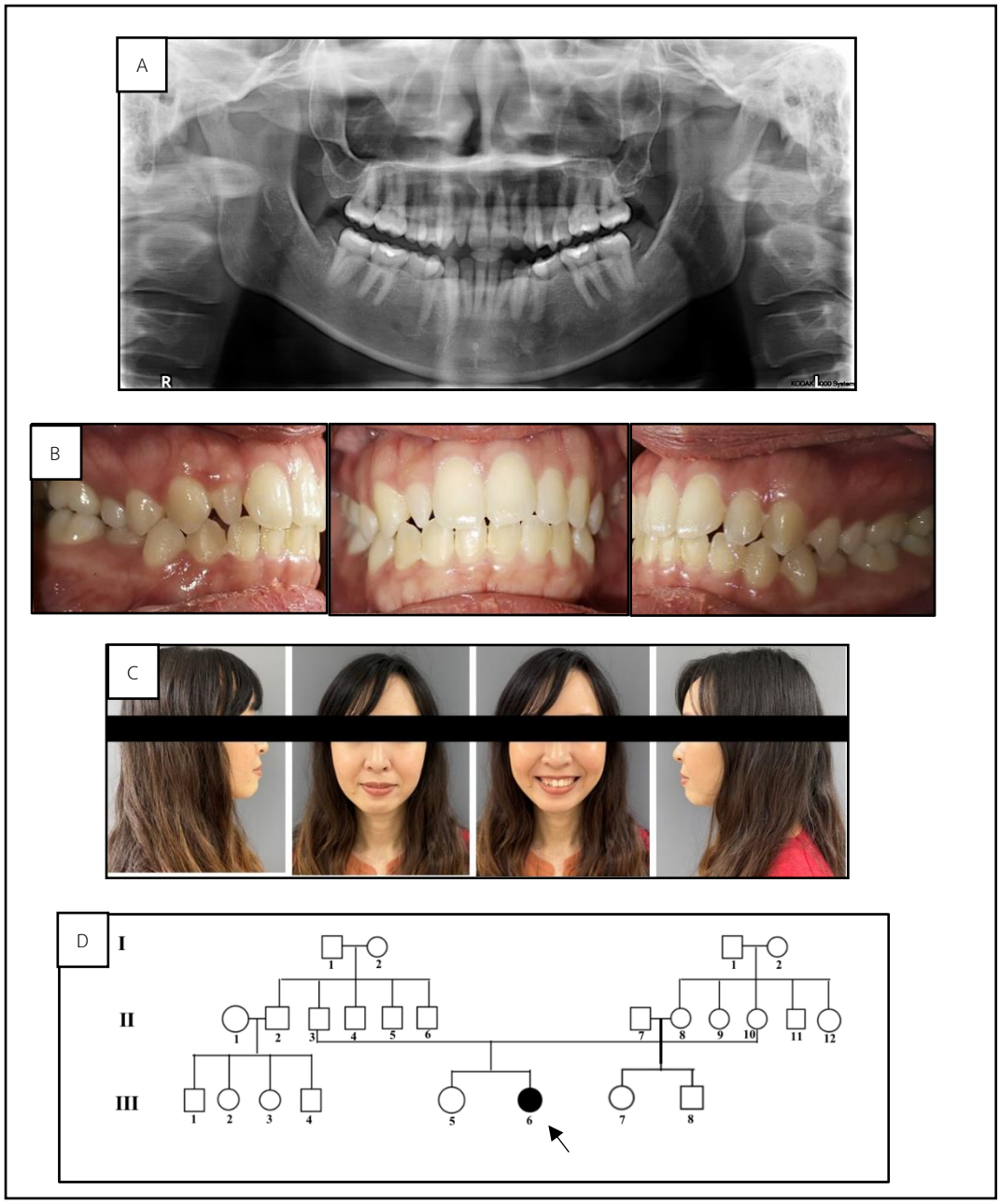


Figure 8 Clinical and radiographic features and pedigree of proband 11.

Panoramic radiograph shows an absence of permanent lower second premolars and prolonged retention of primary lower second molars (A). Intraoral photos show peg-shaped upper left and right lateral incisors (B). Clinical features (C). Pedigree of proband 11; males are identified by squares and females by circles. Filled symbols are marked as affected individuals and clear symbols identified as unaffected individuals. The proband is identified by the arrow (D).

WES detected that the proband possessed the heterozygous missense variant, c.511C>T (p.Arg171Cys) in *WNT10A.* Even though, the proband's mother did not affected with tooth agenesis, the variant c.511C>T (p.Arg171Cys) in *WNT10A* was also detected (Figure 9 and Table 12).

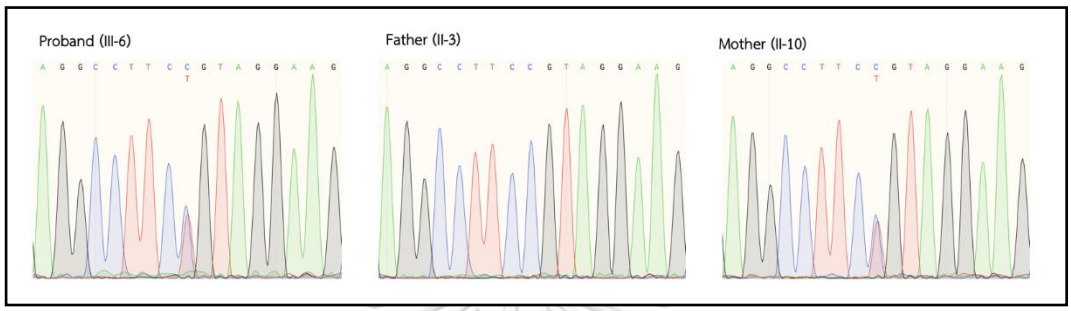


Figure 9 Chromatogram of proband 11's family

Chromatogram of proband 11's family showing the heterozygous duplication, c.511C>T, p.Arg171Cys, in WNT10A.

Table 12 Mutational analysis and characteristics of tooth agenesis of proband 11

Characteristic	Family of Proband 11		
	Proband	Father	Mother
Sex	Female	Male	Female
Missing tooth	Hypodontia	Normal	Normal
Peg-shaped teeth	Yes	No	No
Analysis method	Trio	Trio	Trio
Gene	*WNT10A*	-	*WNT10A*
WNT10A varient	c.511C>T	-	c.511C>T
Protien Change	p.Arg171Cys	-	p.Arg171Cys
Zygosity	Heterozygous	-	Heterozygous
ThWES	69		69

CHAPTER V

DISCUSSION

Tooth agenesis is proven to be one of the most common anomalies in humans. Many studies on the prevalence of tooth agenesis in permanent teeth have been published over the past decades. Studies based on prevalence and distribution of tooth agenesis demonstrated a high variability depending on sample size, gender, race, and ethnicity (28). The latest systematic review by Khaled Khalaf *et al.* (2014) reported the overall prevalence of tooth agenesis to be 6.4% calculated from 93 studies. The study found a statistically significant difference in the prevalence of tooth agenesis by the continent. The prevalence was highest in Africa (13.4%), followed by Europe (7%), Asia (6.3%) and Australia (6.3%) with a lower prevalence in North America (5.0%) and Latin America and Caribbean (4.4%) (2).

Polder *et al. (*2004) study, it appears that the prevalence of tooth agenesis has increased over time in Asia (from 4.7 to 6.3%), Europe (from 5.5 to 7%), and North America (from 3.9 to 5%). from year 1936 to 2002 (15). Studies among Asians reported varieties of the prevalence rates, including 9.4%, 11.2% and 6.9% in Japanese , Koreans, and Chinese, respectively (6).

Until now, the prevalence and pattern of tooth agenesis in Thailand have been demonstrated in only two studies. Tantanapornkul (2015) showed that the prevalence of hypodontia was 13.7% (84 out 638 patients in upper central area of Thailand) and

the most commonly missing tooth was the lower lateral incisor (26.32%), followed by lower premolars (24.81%), and upper lateral incisor (19.55%) (13). Kositbowornchai *et al.* (2010) observed the prevalence at 26.4% (150 out 570 patients) in northeastern area of Thailand. In their study, the lower lateral incisor had the highest prevalence of missing, followed by the upper lateral incisor and lower second premolar respectively (14). Both Thai studies included only orthodontic patients and showed different results. The prevalence of hypodontia reported by Tantanapornkul (2015) was 13.7% which was lower than that reported by Kositbowornchai et al. (2010) at 26.4%. In our study, the prevalence of tooth agenesis was 9.23%, which was lower than other two studies in Thailand. These could be due to the differences of populations included in the studies.

Prevalence by sex

Hobkirk, Goodman and Jones (1994) reported that females were more affected by hypodontia than males in the permanent dentition, but not the primary dentition (16). Polder *et al.* (2006) found the incidence of tooth agenesis in females was 1.4 time higher than that in males (15). In this study the prevalence of tooth agenesis in females (9.95%) was higher than males (8.23%), although the difference between gender was not statistically significant. This was possibly due to lower number of sample size, compared with previous studies.

Prevalence by type of the missing teeth

The permanent third molars are the most commonly absent teeth in the dentition (6). The third molar is the most frequently affected tooth in association with hypodontia and it has been reported that at least one third molar is congenitally absent in 20–30% of the European population (2). However, the third molars are generally excluded from hypodontia studies due to the high frequency of their absence. When the third molar is excluded from studies, the reported prevalence rates for each tooth vary according to the population (4). In Thai population, Tantanapornkul (2015) showed that the most commonly missing tooth was the lower lateral incisor (26.32%), followed by lower premolars (24.81%), and upper lateral incisor (19.55%) (13). Kositbowornchai *et al.* (2010) observed that the lower lateral incisor had the highest prevalence of missing, followed by the upper lateral incisor and lower second premolar, respectively (14).Polder *et al.* (2004) found that an absence of maxillary lateral incisors usually occurred bilaterally, whereas unilateral agenesis was commonly found in the second mandibular premolar (15).

Consistent with previous studies in Thailand, our study showed that the most common missing tooth in the Thai population was the lower lateral incisor. The second and third most common missing teeth in this study were the lower second premolar followed by upper lateral incisor. These are similar to Tantanapornkul (2015) and the study of Chung *et al.* (2007) in Korea, but different from Kositbowornchai *et al*. (2010) (upper lateral incisor and lower second premolar were the second and third common

missing teeth) (14). In other populations, the upper lateral incisors were the most common missing tooth in Turkish, Indian, Mexican and Brazilian populations and the lower second premolar in Japanese population (6).

The maxillary central incisors, mandibular canines, maxillary and mandibular first molars were found to be the least affected teeth (5, 15). Consistently, the maxillary and mandibular first and second molars and mandibular left canines were not found to be absent in our study.

Prevalence by number of missing teeth

The systematic review of Khaled Khalaf *et al.* (2014) found that tooth agenesis of 1 or 2 teeth was the most common (81.6%) followed by tooth agenesis of 3 to 5 teeth (14.3%) and tooth agenesis of 6 or more teeth (3.1%) (2). Similarly, all of 101 patients in our study with tooth agenesis, 43.56% had one missing tooth, 41.58% had two missing teeth, 4.95% had three to four missing teeth, and 5.94% had six or more missing teeth.

Prevalence by location

Tooth agenesis was found more often on the right side (54.09%) than on the left side (45.91%), but the difference was not statistically significant. By dental arches, tooth agenesis was found more in the mandibular arch (61.82%) than the maxillary arch (38.18%). Tantanapornkul (2015) reported the percentage of tooth agenesis in the mandibular arch was higher than maxillary arch (55.64% and 44.36%, respectively). In contrast, Kositbowornchai *et al.* (2010) reported a prevalence of 53.7% in maxillary

arch which was higher than that of 46.3% in mandibular arch (14). Similarly, Khaled Khalaf *et al.* (2014) found a higher percentage of tooth agenesis located in the maxilla (53.2%) compared with 46.8% in the mandible (2). A finding by Polder *et al.* (2004) reported the comparable prevalence of tooth agenesis in the mandible and maxilla (15). These suggest that the location of tooth missing according to the right-left and upper-lower arches are variably found.

The dissimilarities between our study and previous Thai studies could be due to the differences among ethnic groups in Thai populations (North, Northeast parts, and central of Thailand). The ethnicity of the participants, syndromic involvement, and environment factors could be included for future studies to expand the knowledge of tooth agenesis. It is possible that the inclusion criteria, diagnostic criteria, and design of the studies may affect the prevalence of tooth agenesis.

In general, the diagnosis of tooth agenesis in the permanent dentition should be made after the age of 6 years, excluding the third molar, and after 10 years of age if the third molar is included in the study (29). Meta-analysis by Vahid Rakhshan (2015) recommended that the subjects younger than 12-13 years of age should be excluded to avoid the possibility of delayed tooth development and both sexes should be equally included (6). It was also suggested that the study should not include only the orthodontic patients as the patients having tooth agenesis were more likely to seek orthodontic treatment which could bias the prevalence of tooth agenesis. We noticed

that both previous two studies in Thailand reported the prevalence of tooth missing in orthodontic patients. Instead, our study examined general dental patients, not only the orthodontic ones. The prevalence found in our study could therefore be a valid prevalence of tooth agenesis in Thailand. More sample included in future studies could validate our findings.

Variant identification

Mutations in *WNT10A* are found in 3 out of unrelated 11 probands affected with tooth agenesis (27.27%, n= 3). The first one had 9 missing teeth, the second had 8 missing teeth, and the third had 2 missing teeth (Table 9).

In this study, the first proband was identified with the heterozygous duplication, c.916_918dupAAC (p.Asn306dup) in *WNT10A*. The second proband possessed the homozygous missense variant, c.511C>T (p.Arg171Cys) in *WNT10A* and the heterozygous missense variant, c.413A>T (p.Asn138Ile) in *EDARADD*. The third proband harbored the heterozygous missense variant, c.511C>T (p.Arg171Cys) in *WNT10A*. However, both *WNT10A* variants, c.511C>T (p.Arg171Cys) and c.916_918dupAAC (p.Asn306dup) were also found in unaffected family members, suggesting that *WNT10A* variants could be incomplete penetrant.

The majority of *WNT10A* mutations that have been reported are found in European origin, such as Germany, France, Poland, and the Netherlands (26). From Netherlands origin, *WNT10A* gene mutations have been reported in 55.9 % of isolated

oligodontia cases (agenesis of between 6 and 28 teeth) and 62 % of severe tooth agenesis cases (agenesis of between 4 and 26 teeth) from Polish origin (24). The study concluded that among all tooth agenesis cases including non-syndromic mild tooth agenesis, non-syndromic severe tooth agenesis, and syndromic severe tooth agenesis, *WNT10A* variants should be most frequently present in non-syndromic severe tooth agenesis cases (30).

The type of missing teeth in Japanese severe tooth agenesis patients (at least 4 missing-tooth) with *WNT10A* variants varies from those in the study in Chinese patients (26). The absence of the maxillary lateral incisors was higher in Japanese patients (63.6%) than Chinese with severe tooth agenesis (31.3%) (30). According to the observations of Nieminen (2009), the position and number of missing teeth were related to specific gene mutations (31). Arzoo *et al.* (2014) concluded that mutations in *WNT10A* correlated with increased premolar agenesis (31). Conversely, Mostowska *et al.* (2015) indicated that variations in *WNT10A* were responsible for maxillary lateral incisor agenesis in the Polish population (32). According our observations, the absence of lower premolars were found all 3 probands with *WNT10A* variants.

Shujuan Song *et al.* (2014) showed that the two common *WNT10A* variants, c.511C>T and c.637G>A, accounted for 78.6 % of all variants in Chinese patients, and were considered to be associated with tooth agenesis (26). Three *WNT10A* variants (c.1039G>T, c.862-863insG, and c.1052-1053delGC) were considered pathogenetic in

previous study (26). Furthermore, In the Chinese patients with at least 4 missing teeth, the *WNT10A* variants c.511C>T (p.Arg171Cys) and c.637G>A (p.Gly213Ser) were frequently detected (22.6%; 7/31 and 25.8%; 8/31 respectivly) (26).

The study of Machida *et al.* (2017) showed the prevalence of *WNT10A* variants in the Japanese patients with tooth agenesis of more than three teeth was lower than that of other reports in other ethnic population (30). The prevalence of *WNT10A* variants ranged about 30–50% among different ethnic groups. 22% of 50 Japanese patients lacking at least 4 teeth excluding wisdom teeth were identified with missense mutation in *WNT10A* whereas 0% was found with the *WNT10A* variants in the healthy 50 Japanese controls (30). Of those, the c.511C>T variant was detected in one patient, the c.637G>A variant was found in 16% of tooth agenesis patients. These two *WNT10A* variants were not found any healthy Japanese controls. According to the study of Song *et al.* (2014), showing the data from the Human Genetic Variation Database, the population ratios of c.511C>T and c.637G>A *WNT10A* variant were 3.1% and 3.0%, and the allelic frequencies were 0.0147, and 0.0149, respectively, indicating that these two *WNT10A* variants can be frequently found in general populations who might not have tooth agenesis (26). The c.511C>T and c.637G>A *WNT10A* variants were detected in normal Chinese control at 2.0% and 2.7%, respectively (26). In addition, the heterozygous *WNT10A* variants were identified in unaffected individuals in tooth agenesis families, as well as in unrelated control individuals with no tooth agenesis or

family history of tooth agenesis. Approximately 41% of individuals showed a single heterozygous variant in *WNT10A* did not have tooth agenesis (21).

Our study found the allelic frequency of c.511C>T variant was 0.016 (69/4330) or 1.6% in our in-house database of Thai population (THWES). These indicate that c.511C>T variant can be frequently found in general population who might have or not have tooth agenesis. More Thai patients with tooth agenesis should be recruited for genetic study to validate the pathogenicity and causative effects of the *WNT10A* variants. It is likely that tooth agenesis phenotypes might be overlooked in the database samples, since tooth agenesis is rather mild and could not be noticed by affected individuals or medical practitioners compared with other serious congenital disorders.

CHAPTER VI

CONCLUSION

This study reports lower prevalence of congenital missing teeth (9.23%) compared to previous studies in Thai population, but higher compared to the overall prevalence from previous meta-analysis. This suggested that tooth agenesis is a common anomaly in Thai population. A single tooth absence is most common and according to types of tooth, the mandibular second premolar is the most frequently missing tooth.

We identify 3 probands affected with tooth agenesis who have either homozygous or heterozygous variants in *WNT10A*. Biallelic genotypes of *WNT10A* variants may have a pathogenic effect on tooth development. Presence of a single variant allele would be a predisposing factor to tooth missing with incomplete penetrance. In addition to the *WNT10A* variants, other genetic or environmental factors might contribute to the variability of clinical manifestations. The role of the *Wnt10A* variants, c.511C>T (p.Arg171Cys) and c.916_918dipAAC (p.Asn306dup), as a single causative factor for tooth agenesis could not be determined in our study since this *WNT10A* variants are not segregated with tooth agenesis phenotype in the families.

An early detection of missing teeth by careful clinical and radiographic examinations would minimize the complications of missing teeth and restore the patient's esthetics and masticatory function at the earliest time and the most beneficial way. Further studies of molecules and cellular mechanisms in tooth

development may provide more clues to understand the genetic cause and pathomechanism of tooth agenesis.

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VITA

NAME	Charinya Kanchanasevee
DATE OF BIRTH	25 May 1990
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Chulalongkorn University
HOME ADDRESS	3 Soi Assawin 1 Charunsanitwong 47 Road Arunamarin Bangkoknoi Bangkok